

reporter. Using the second methods we found out that the UCS domain but not the central domain prevents the thermal aggregation of the myosin motor domain. We conclude that while both the UCS domain and the central domain bind the myosin head, only the UCS domain displays chaperone activity. Funded by a grant from the American Heart Association (AHA 13GRNT17290006).

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Poly-(R)-3-Hydroxybutyrate-Modified Proteins

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Poly-(R)-3-hydroxybutyrate (PHB), a linear polymer of R-3-hydroxybutyrate (R-3HB), is a universal constituent of biological cells. Short-polymers of PHB (<15 residues) are covalently attached to certain prokaryotic and eukaryotic proteins (PHBylation) - primarily proteins of membranes and organelles. PHB is an amphiphilic, water-insoluble, lipid-soluble molecule that may form hydrophobic, hydrogen and coordinate bonds. The PHB backbone is highly flexible at physiological temperatures but becomes increasingly more rigid as temperatures are lowered. These physical properties indicate that PHBylation plays important roles in the targeting, folding, structure and function of PHB-modified proteins, and also promotes interactions between proteins and polyanions such as RNA, DNA and inorganic polyphosphate (polyP).

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GFP Variants with Alternative Strands: Protease Sensor Design and their Thermodynamic Analysis

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Green fluorescent protein (GFP) variants with one extra strand 10 (s10) can fold with either one or the other s10, and the composition of the two bound forms can be unambiguously distinguished by their resulting colors (Do & Boxer, 2013 JACS). The composition can be systematically modulated by mutating the residues on s10 or by changing the lengths of the two inserted linker sequences that connect each s10 to the rest of the protein. We have applied thermodynamic analysis on the composition to obtain insight into folding of the variants, and discovered robust empirical rules that accurately predict the product ratios of a given construct. Ratiometric protease sensors were designed from the construct by recombinantly adding a protease cleavage sequence into one of the inserted loops, where the bound s10 is replaced by the other upon protease cleavage and irradiation with light, which is based on a photodissociation phenomenon previously reported (Do & Boxer, 2011 JACS). Since the conversion between the two bound forms involves a large spectral shift, the sensor can display a very high dynamic range. The variants can serve as a platform to provide useful insight into protein folding in general, and further engineering of this class of proteins will enable their application as light-dependent modulators.

Enzyme Function and Regulation

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Steered Molecular Dynamics Simulations of NAD Unbinding from GAPDH and LDH

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Protein-ligand interactions play an important role in understanding biophysical processes including the glycolytic pathway. Calculation of the energy profile of ligand unbinding is essential for understanding possible substrate channeling of nicotinamide adenine dinucleotide (NAD) between lactate dehydrogenase (LDH) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Herein, steered molecular dynamics (SMD) simulations elucidate the process of NAD unbinding from LDH and GAPDH. Brownian dynamics (BD) simulate, using the energy reaction criterion, NAD diffusion towards the binding site of GAPDH or LDH to identify potential residues where strong protein-ligand coulombic interactions exist. These residues are used to design several dissociation pathways for the SMD simulations. Simulations either apply a harmonic guiding potential or a constant force SMD to perform center of mass (COM) pulling of the NAD. The two ligands in the tetrameric GAPDH protein are successfully released from the binding pocket using a force constant $k \geq 5000$ kJ/mol/nm² or a constant force $F \geq 600$ pN, within the first 4.2 ns of simulation time. A constant force of 600 pN is enough to pull out three of the four ligands from their corresponding LDH binding sites within the first 0.5 to 1.2 ns of simulation time. Upon releasing the ligand from the binding site, NAD conformational changes are traced, starting with a stretched, open conformation in the

binding site and ending with a bent structure in solution. The bent structure is consistent with previous experimental and simulation data of NAD free in solution. The unbinding free energies associated with the NAD release along the proposed pathways are calculated using the Jarzynski equality, in the stiff-spring approximation of pulling.

3416-Pos Board B144

Computational and Experimental Study of Ketoreductase Enantioselectivity

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Enzymes are powerful enantio-, chemo-, and regioselective catalysts. They are highly efficient, operate under mild conditions, and are environmentally benign. These attributes make them great synthetic tools for chemical manufacturers. Ketoreductases (KREDs) asymmetrically reduce ketones to alcohols and are the most commonly used enzymes in industrial drug synthesis. Various mutants of *L. Kefir* KRED have been generated to enantioselectively reduce the substrates dihydrothiophen-3(2H)-one and dihydrofuran-3(2H)-one, and the alcohols produced can be incorporated into pharmaceuticals such as sulopenem and fosamprenavir. These substrates vary by only one atom, sulfur versus oxygen, and yet in general the KRED mutants reduce them to different enantioselectivities. In many cases mutants that produce good to excellent enantiomeric excess (ee) for one substrate have no enantioselectivity for the other. Other mutants give opposite enantioselectivities for the two substrates. In this work, the physical origins of this subtle and perplexing example of specificity are determined using several quantum mechanical and force field based computations. These methods reveal that small changes in the size, shape, and hydrophobicity of the active site resulting from mutations in as few as 1 residue can significantly effect enantioselectivity. Furthermore, the ability of various enzyme design methods to predict selectivity is evaluated through comparison to experimentally determined selectivities. This work will enable further development of KRED mutants for industrial drug synthesis and aid enzyme design efforts.

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In Support of Nitric Oxide Dioxygenase Function: Algal Hemoglobins and their Reduction Partners

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The ubiquity of hemoglobins as a superfamily to life has enthused the field with renewed vigor. Reactions like oxygen binding and nitric oxide (NO) dioxygenation appear to be characteristic to the hemoglobin superfamily, as revealed from investigation of recombinant globins, irrespective of whether they are associated to any particular function like oxygen transport/storage, sensing, electron transport, protection against hypoxia and other possibilities. **NO dioxygenase** reaction, common in vitro, however, was limited by lack of report of specific enzymes that can convert ferric hemoglobin, formed during reaction of oxy hemoglobin with NO, into ferrous hemoglobin - the species that reacts with NO. Absence of a known cognate reductase would prevent reduction of ferric species of hemoglobin to ferrous form and the oxidation-reduction cycle would be incomplete for NO related function to be fruitful. Assignment of NO dioxygenase activity as a physiological function requires the design of experiments that address reduction mechanisms. We used *Chlamydomonas reinhardtii* as model system since we have identified 12 globins and 3 putative genes that can potentially function as reductase of ferric hemoglobin. Organism database annotated these reductases as **dihydrolipoamide dehydrogenase**, **cytochrome b5 reductase** and **monodehydroascorbate reductase**. So far, we have characterized 3 hemoglobins and 3 putative cognate reductases using biochemical and biophysical methods. Spectroscopic studies reveal that *Chlamydomonas* contains both pentacoordinate and hexacoordinate hemoglobins. The enzymes were found to contain flavin domain and could reduce ferric *Chlamydomonas* hemoglobins *in vitro* to their functional ferrous state. The interactions between hemoglobins and these reductases might support NO scavenging/detoxification function of globins with potential implications in biotechnology.

3418-Pos Board B146

Mapping the Substrate Binding Sites of the Integral Membrane Methyltransferase ICMT by Mutational Analysis

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The eukaryotic enzyme isoprenylcysteine carboxylmethyltransferase (ICMT) catalyzes the attachment of a methyl group onto the carboxylate of a lipid-modified cysteine at the C-terminus of its protein substrates. This is the final